Mycotoxins in the Indoor and Outdoor Environment

American Academy of Clinical Toxicology
Occupational and Environmental Symposium

Mycotoxins: Exposure Sources, Pathways, and Clinical Toxicology

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Presentation Overview

- Aflatoxins as an example of advances in clinical toxicology
  - Toxicokinetics, biomarkers of exposure
    - Recent developments in chemoprevention of cancer
- Trichothecenes as mycotoxins of agricultural importance, and historical importance
  - Current challenges relating to exposure and risk assessment, clinical toxicology

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Aflatoxins

- More extensively studied than other fungal metabolites
- Historical significance
  - Turkey X disease
    - 1960, England
    - Thousands of farm animal deaths
    - Disease traced to contamination of peanut meal
      - Aflatoxins produced by *Aspergillus flavus*
- Human health significance
  - Acute high-level dietary exposures resulting in hepatic injury, failure (aflatoxicosis)
  - Chronic low-level dietary exposure as a risk factor for liver cancer in some areas of the world

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Toxigenic Fungi and Aflatoxins

- Aflatoxins produced by certain *Aspergillus* species
  - *A. flavus*
  - *A. parasiticus*
- Both species found worldwide
  - Ambient air
    - Indoors and outdoors
  - Plant pathogens
    - Corn, nuts
  - Post-harvest contaminants of agricultural commodities
    - Cottonseed, tobacco

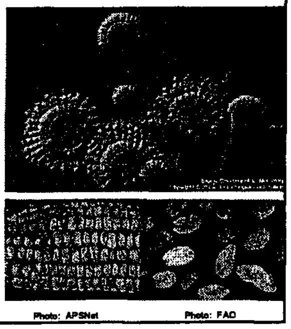


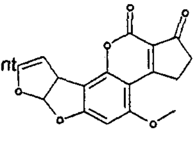
Photo: APSNet Photo: FAO

Toxigenicity of *Aspergillus flavus, Aspergillus parasiticus*

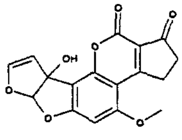
- Some strains do not produce aflatoxins
- Factors affecting aflatoxin production
  - Growth substrate (moisture, nutrient content)
  - Temperature
  - Humidity
  - Growth of competing microorganisms
    - Can inhibit aflatoxin production
      - *Phoma spp.*
    - Can metabolize and detoxify aflatoxin
      - *Trichoderma spp., Rhizopus spp., Alternaria spp., Mucor spp., A. flavus*

Aflatoxins

- Difuranocoumarin derivatives
- 18 structural variants identified
- Aflatoxin B_1 (AFB_1) as most potent compound
  - IARC Class I carcinogen
- Aflatoxin M_1 (AFM_1) as hydroxylated metabolite
  - considered a detoxification product of AFB_1
    - Can be transferred into animal-derived foods
      - Meats
      - Milk
- Heat-stable compounds
- Non-volatile



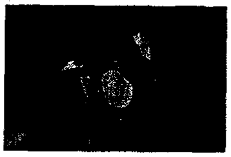
Aflatoxin B_1



Aflatoxin M_1

Aflatoxins in the Environment

- Raw agricultural commodities
  - Unavoidable contaminants
  - FDA action level for aflatoxins
    - 20 ppb as AFB_1
    - .5 ppb as AFM_1 in milk
- Airborne grain dust (agricultural environments)
  - <<ppt concentrations AFB_1 measured during grain handling activities
- AFB_1 has not been detected in air samples or surfaces of residential or other occupational environments in studies of building-related illness

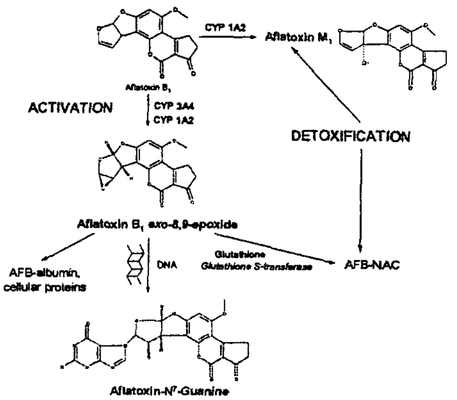


AFB_1 fluorescence under UV light

Farmer cleaning grain silo

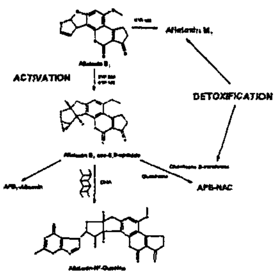
Aflatoxin Toxicology: An Overview

- Oral bioavailability of AFB_1 is not well-understood
- Significant first pass metabolism occurs
  - Responsible for target organ effects in liver
- Metabolism of AFB_1 can result in the formation of reactive intermediates
- Reactive intermediates are a critical step in cytotoxic and genotoxic pathway



Elimination of Aflatoxins

- Relative role of biliary and urinary elimination is not clear in humans
- AFB_1 is rapidly cleared from the blood
  - $t_{1/2}$ < 90 minutes
- Short (<8 hours) elimination half-life for aflatoxin-N^7-guanine adducts, AFB-NAC, AFM_1
- Longer elimination half-life (2-3 months) for AFB-albumin adducts

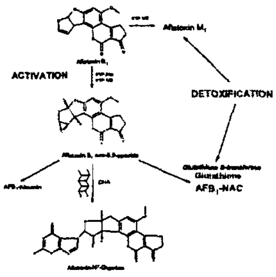


Acute Aflatoxicosis: Clinical Effects

- Result from reactive intermediates on hepatocellular enzyme function
- Hepatocellular injury begins in zone 3, extends to zone 1
- Clinical course
  - Abdominal pain, distention, anorexia, vomiting
  - Coagulopathy, metabolic acidosis, encephalopathy, massive GI bleeding
  - Case fatality rate ranging from 10-70%

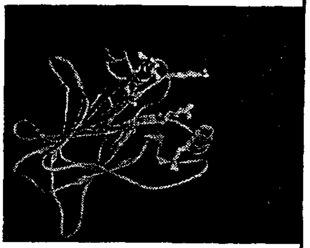
Acute Aflatoxicosis: Clinical Management

- Supportive
- Antidotes have not been investigated in humans
  - Early role for N-acetylcysteine?
  - Some evidence for efficacy in a recent experimental animal study
    - Valdivia AG, 2001
- Transplantation has not been reported



Toxicodynamics of Chronic Dietary Aflatoxin Exposure

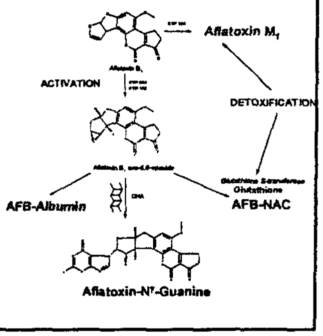
- Genotoxic effects
- Aflatoxin-N^7-guanine adducts can give rise to transversion mutations in DNA
  - Guanine→Thymine
- High frequency of this mutation has been observed at the p53 tumor suppressor gene
  - Codon 249 as mutational "hot spot"
    - DNA-binding region of protein

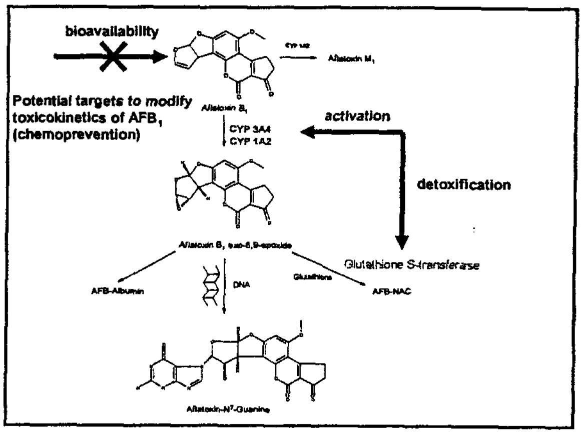


p53 protein interacting with DNA

Biomarkers of Dietary Aflatoxin Exposure

- Sensitive and specific indicators have been developed
- **AFM$_1$** in urine (biomarker of *exposure*)
  - Correlates with recent ingestion
- **AFB-N^7-Guanine** adducts in urine (biomarker of *exposure and genotoxic effect*)
- **AFB-NAC** (detoxification product) in urine
- **AFB-albumin** adducts *in serum*
  - Indicator of chronic dietary exposure
- Validation of biomarkers has led to search for agents that *modify* toxicokinetics
  - Chemoprevention





bioavailability

Potential targets *to modify* toxicokinetics of AFB$_1$ (chemoprevention)

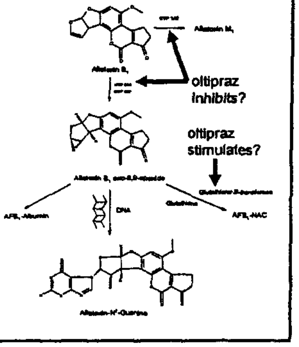
activation

detoxification

Glutathione S-*transferase*

Oltipraz and Dietary Aflatoxin

- Antischistosomal drug
  - No longer used in U.S.
- Pharmacodynamics
  - Induction of phase 2 enzymes (GST)
  - Inhibition of *CYP450*
    - CYP1A2 and 3A4
- *Animal* studies (oltipraz and dietary aflatoxin)
  - Lowered levels of aflatoxin *biomarkers*
  - Lowered incidence of HCC
- An **investigational** study drug for chemoprevention of *human* cancers

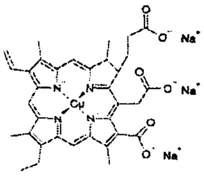


oltipraz *inhibits?*

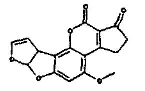
oltipraz stimulates?

Other Chemoprevention Agents for Dietary Aflatoxin exposure

- Chlorophyllin
  - Water-soluble salt derived from natural chlorophyll
  - Derivation involves replacement of central *Mg atom* with Cu
- Anti-mutagenic properties *in vitro* and *in vivo* (animals)
  - Forms tight non-covalent molecular complexes with carcinogens
  - **Decreases oral bioavailability**
- An **investigational** drug for human chemoprevention studies



Chlorophyllin



Aflatoxin B$_1$

Chemoprevention Trials of Oltipraz, Chlorophyllin (Qidong, China)

- Randomized, double-blinded, placebo-controlled trials
- Intervention periods of 8 weeks-4 months
- Significant effects on aflatoxin toxicokinetics reported in both studies
  - Oltipraz increased excretion of AFB-NAC (detoxification product)
  - Chlorophyllin decreased excretion of AFB-N^7 guanine adducts (biomarker of genotoxic effect) in urine

Dietary Aflatoxin and Chemoprevention Trials

- Provide evidence of principle
  - Interventions may favorably alter toxicokinetics of dietary aflatoxin exposure
- Questions in need of further research
  - Can these interventions be implemented on a population-basis?
    - Altered kinetics of other drugs
  - Can altered toxicokinetic effects be sustained over time?
  - Does intervention alter risk of HCC?

Aflatoxin Summary

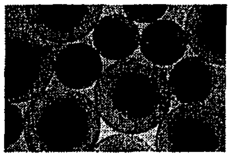
- Toxigenic fungi producing aflatoxins (*A. flavus, A. parasiticus*) are ubiquitous
- Production of aflatoxins difficult to predict
  - Influence of specific strain and environmental factors
- Diet represents the most common pathway of human exposure to aflatoxins
- Human disease results from bioactivation pathways leading to cytotoxic and genotoxic effects
- Interventions to alter toxicokinetics of dietary aflatoxin are currently under investigation for cancer prevention in high-risk populations

Trichothecene Mycotoxins

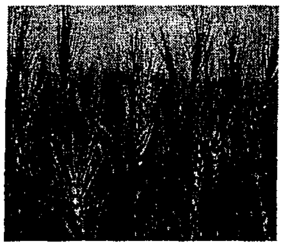
- Family of structurally-related mycotoxins
  - Varying potency
- Produced by several species of toxigenic fungi
  - Found throughout the world
- Agricultural importance
  - Unavoidable contaminants of food
- Historical importance
  - Human foodborne epidemics, chemical warfare
- Risk assessment: the indoor environment
  - Building-related illness?

Toxigenic Fungi and Trichothecene Mycotoxins

- *Fusarium graminearum*
  - Pre-harvest plant pathogen
    - Deoxynivalenol
- *Fusarium poae, Fusarium sporotrichioides*
  - Post-harvest contaminant
    - T-2 toxin



Fusarium graminearum strains



Fusarium head blight, caused by *Fusarium graminearum*

Trichothecenes and Toxigenic Fungi: Other Species

- *Myrothecium roridum*
  - Common soil fungus
  - Macrocyclic trichothecenes
- *Stachybotrys chartarum*
  - Common soil fungus
  - Water-damaged buildings
- *Trichoderma spp.?*
  - <1% found to produce trichothecenes (Nielsen KF, 2001)
  - Certain species have been registered by EPA as biopesticides
    - Competitive inhibitors of toxigenic, pathogenic fungal growth on food crops

***S. chartarum* growing on recycled paper**

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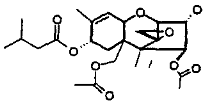
Dill, et al., 1997. Mycoses 40:110-114

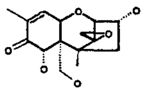
Production of Trichothecenes by Toxigenic Species

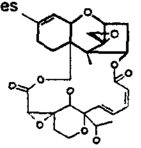
- Factors affecting trichothecene production
  - Strain specificity (genetics)
  - Environmental conditions of their growth
    - Temperature, humidity, growth substrate, growth of other competing microorganisms
- The presence of a toxigenic fungal species is not an accurate indicator of trichothecene production
  - A frequently misunderstood concept

Trichothecene Mycotoxins

- Characterized by sesquiterpenoid ring *structure*, double bond at $C_{9,10}$ position, epoxide-group at $C_{12,13}$ position
- Resistant to degradation by light, heat
  - Deactivated under strong acid or alkaline conditions
- Non-volatile compounds
- Environmental fate difficult to *predict*
  - *Alternaria sp., Trichoderma sp., Cladosporium cladosporioides,* bacteria capable of metabolizing trichothecenes


Group A: T-2 toxin

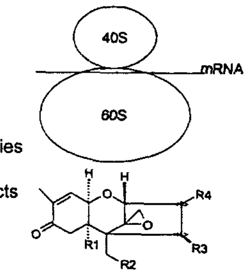

Group B: deoxynivalenol


Macrocyclic: satratoxin G

Trichothecenes: Potential Exposure Sources and Pathways

- T-2 toxin in agricultural commodities
  - Corn, wheat, oats, barley, *rice*
  - WHO: estimated human intake of T-2 toxin =8 ng/kg body weight/day
    - LOEL 29 *mcg/kg body* weight/day
- Deoxynivalenol in agricultural commodities and commercial foods
  - Wheat, corn, barley, *other grains*
  - Beer, breakfast cereals, bread, rice
  - WHO: estimated human intake of deoxynivalenol = .77 – 2.4 mcg/kg body weight/day
    - NOEL 100 mcg/kg body weight/day
- Trichothecenes in moldy building materials
  - nanogram quantities reported *on* surfaces and in bulk samples
  - Trichothecenes have not been quantified in indoor air, in epidemiological studies of building-related illness

Trichothecene Toxicology



- *Early* toxicodynamic studies
  - Inhibition of protein synthesis
  - Binding to 60S subunit
  - Inhibiting activity of *peptidyltranferase*
    - Interfering with initiation or elongation phase of protein synthesis
- More *recent* mechanistic studies *in vitro* and *in vivo* suggest additional toxicodynamic effects
  - Lipid peroxidation
  - Apoptosis
  - Immunomodulatory effects

Trichothecenes and Human Toxicology

USDA ARS Photo

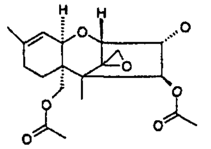
- Epidemic of food-borne *illness*
- Former Soviet Union, 1930's-1940's
- Conditions of near-famine
- Consumption *of over-wintered* grain
  - Contaminated with *F. poae, F. sporotrichioides*
  - Strains subsequently found to produce high levels of T-2 toxin
- Repeated exposure associated with a clinical syndrome leading to thousands of fatalities
  - Alimentary toxic aleukia (ATA)

Clinical Stages of Alimentary Toxic Aleukia (ATA)

Stage	Symptoms	Signs
1 (days)	Vomiting, diarrhea, abdominal pain	Mucosal hyperemia
2 (days-weeks)	Symptoms less prominent (latent stage)	Bone marrow suppression: Leukopenia, anemia, thrombocytopenia
3	Odynophagia, aphonia, shortness of breath	Necrotic lesions of GI tract, upper airways *Progressive* bone marrow suppression Hemorrhagic and infectious complications
4	Convalescence (recovery)	Resolution *of necrotic* lesions, hematologic abnormalities (leukocytes, followed by granulocytes, platelets, and *erythrocytes*)

Trichothecenes and Human Pharmacology

- Chemotherapy trials for advanced malignancies
- Phase I clinical study
  - No drug-related toxicity at daily intravenous doses <2.4 mg/m² x 5 consecutive days (Murphy, 1978)
  - Dose-dependent adverse effects reported at higher dosages
  - Gastrointestinal symptoms, myelosuppression, hypotension, transient neurological symptoms
- No pharmacokinetic studies done
- Efficacy not demonstrated
  - Evaluation of did not proceed beyond Phase II stage



Anguidine (diacetoxyscirpenol)

Trichothecenes as Chemical Warfare Agents (T-2 Toxin)

- 1980: Allegations that Soviet Union and allies used T-2 toxin in Southeast Asia on refugee populations
  - Laos, Kampuchea, Afghanistan
  - Various symptoms: vomiting, dizziness, chest pain, skin burning, seizures, hematemesis
  - "Yellow Rain"
    - Trichothecenes or bee pollen?
- Allegations remain contentious
  - U.N. investigation: evidence was inconclusive that any chemical or biological agents had been used
- Led to further research on toxicology of T-2
  - Experimental animal models

T-2 Toxin and Animal Toxicology, Dermal Exposure

- Many studies conducted in context of chemical warfare investigation used DMSO as carrier
  - Wide range of observed responses by species
    - Mouse: LD_{50} of 5 mg/kg
    - Swine: local injury with minimal systemic absorption at 15 mg/kg
- Studies conducted with T-2 alone (no DMSO)
  - Low rate of penetration through skin
  - Irritant effects at doses up to 2.6 mcg / cm^2
    - Threshold for irritant effects ~ .1 mcg / cm^2
- Skin effectively decontaminated using aqueous soap solution, Polyethylene glycol (PEG) 300

T-2 Toxin and Animal Toxicology, Inhalation Exposure

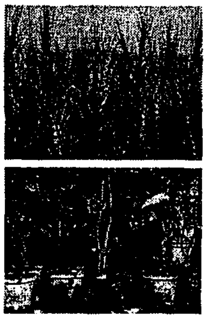
- Target organ effects as consistent finding
  - Spleen, lymphatic tissue necrosis
  - Gastrointestinal tissue necrosis
  - Pulmonary findings *not* prominent
- Some conclusions inconsistent across studies
  - Relative potency of different exposure pathways
    - Inhalation vs. parenteral, oral, or other pathways
- Methodologies vary from study to study
  - Different species (mice, rats, guinea pigs, swine)
  - Different methods of T-2 administration

Trichothecenes and Animal Toxicology, Ingestion Exposure

- Poor oral bioavailability
  - Physiological instability, role of gut flora
- Considerable first-pass metabolism
  - Major *metabolic pathways* include hydrolysis, oxidation, de-epoxidation, glucuronidation
- Instability of T-2 toxin in whole blood
  - Effect of carboxylesterases
- Rapid clearance
  - $t_{1/2}$ ~minutes to hours, depending upon species
- Biliary and urinary elimination
  - Enterohepatic recirculation in some species

Extrapolating Experimental Studies to Environmental Exposure

- *Difficult*
  - Not reflective of environmental levels or exposure conditions
- Dietary exposures to trichothecenes
  - Human dose estimates are lower than animal NOEL's
- Indoor exposures to trichothecenes
  - Non-detectable to low levels on moldy surfaces, water-damaged building materials
  - No quantification in indoor air
  - Hazard and exposure assessment in studies of BRI have focused on toxigenic fungi, not trichothecenes

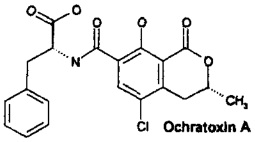


Challenges to the Clinical Toxicologist

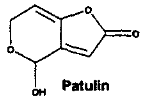
- Data gaps relating to metabolic fate, and elimination for trichothecenes in humans
  - T-2 toxin, deoxynivalenol
    - Limited information
  - Macrocyclic trichothecenes
    - No information
- Development and validation of biomarkers of trichothecene exposure in humans
  - Sensitive analytical methods (GLC, TLC, HPLC, RIA) have been used to study trichothecene toxicokinetics in animal studies
  - Sensitive GC-MS methods have been described for trichothecenes in human blood, urine specimens
    - Have not been utilized, validated in epidemiological studies

Mycotoxins in the Indoor and Outdoor Environment

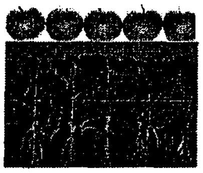
- Aflatoxins as example of advances in understanding the clinical toxicology of mycotoxins
- Trichothecenes as example of an important mycotoxin where human data set is far less complete
  - Hazard and exposure assessment
  - Clinical toxicology

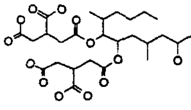


Ochratoxin A



Patulin





Fumonisin B_1

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